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ADVANCES IN CICHLID RESEARCH V

Molecular phylogeny and taxonomic revision of the cichlid genus *Hemichromis* (Teleostei, Cichliformes, Cichlidae), with description of a new genus and revalidation of *H. angolensis*

Anton Lamboj · Stephan Koblmüller

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Abstract The tribe Hemichromini is an early diverging, mainly Central and West African lineage within the species-rich African cichlid fishes (Cichliformes, Cichlidae) including two genera, *Hemichromis* Peters 1858 and the monotypic *Anomalochromis* Greenwood 1985. Though many of the species are popular aquarium fish, the number of hemichromine species is still a matter of debate with their phylogenetic relationships largely unknown. Based on DNA sequence data of two mitochondrial and two nuclear genes, we present the first comprehensive phylogeny of the Hemichromini. Using an integrative approach based on these DNA sequences data, morphometrics, meristics, and a qualitative assessment of body coloration, we revise the genus *Hemichromis* and discuss intrageneric relationships. Two major groups within the genus *Hemichromis* that diverged roughly 6–12 MYA are recognized, of which the first one represents *Hemichromis* sensu stricto, for the second one a new genus, *Rubricatochromis*, is described. Diversification with these two main groups started about 3–6 MYA, with different trajectories of colonization in the two groups. *Hemichromis* populations from the most southern (Cuanza, Zambezi, and Okavango) part of the genus' distribution range constitute a well-supported clade distinct from all other members of *Hemichromis*, for which the taxon *H. angolensis* Steindachner, 1865 is confirmed.

Keywords Hemichromine cichlids · Phylogeography · Revision · *Rubricatochromis* gen. nov.

Abbreviations

SL	Standard length
HL	Head length
AMNH	American Museum of Natural History, New York
BMNH	British Museum (Natural History), London
MNHN	Musèum National d'Histoire Naturelle, Paris
MRAC	Musée Royal de l 'Afrique Centrale, Tervuren
NMW	Naturhistorisches Museum Wien

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A. Lamboj (✉)
Division Integrative Zoology, Department of Evolutionary Biology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria
e-mail: anton.lamboj@univie.ac.at

S. Koblmüller
Institute of Biology, University of Graz, Universitätsplatz 2, 8010 Graz, Austria

SAIAB	South African Institute for Aquatic Biodiversity
USNM	Smithsonian Institution, National Museum of Natural History, Washington
UVZC	University Vienna Zoological Collection
ZMHU	Zoologisches Museum der Humboldt Universität Berlin
ZSM	Zoologische Staatssammlung München

Introduction

With currently 1746 valid species (Fricke et al., 2022), and many more still to be described, cichlid fishes (Cichlidae) are among the most species-rich fish families. Most of their diversity is found in the East African rift lakes, with up to several hundreds of species endemic to each of these lakes (Turmer et al., 2001; Salzburger et al., 2014), making these lakes' cichlid species flocks well-established model systems in evolutionary biology research (e.g., Salzburger, 2018). Even though not as species rich as the East African rift lakes, the Central and West African rivers and lakes are also inhabited by a stunning cichlid diversity, usually from early diverging lineages, whose actual diversity, evolutionary history, and phylogenetic relationships have received increasing attention only fairly recently (e.g., Schwarzer et al., 2011, 2015; Dunz & Schliewen, 2013; Stiassny & Alter, 2015).

One of these Central and West African cichlid lineages is the genus *Hemichromis*, Peters 1858. Together with the monotypic genus *Anomalochromis*, Greenwood 1985a, b, it constitutes the tribe Hemichromini (Greenwood, 1985a, b), which split from the other African cichlid tribes about 38–60 MYA (Matschiner et al., 2017; Irisarri et al., 2018; Schedel et al., 2019). The genus *Hemichromis* was described by Peters (1858), based on specimens of *H. fasciatus*, characterized by a notably protractile praemaxilla and a simplified buccal dentition, consisting of a single row of unicuspid teeth in the lower jaw and a single outer row with an incomplete second row of identical teeth in the upper jaw. The type locality was given in a somewhat unprecise way, as 'Africa occidentalis, Guinea,' together with a label 'Goldküste' (=Gold Coast) on the type specimens. Subsequent descriptions of cichlids belonging to the genus *Hemichromis*, or revisions (e.g., Gill, 1862; Günther, 1862), added several species and put great emphasis on the shape of the buccal teeth in the generic definition.

In 1915, Boulenger revised the genus (Boulenger, 1915). Species with cycloid scales and a well-developed hanging pad on the roof of the pharynx were placed in the genus *Pelmatochromis*, species with ctenoid or granular scales in the genus *Paratilapia*. *Hemichromis* was limited to species with clearly cycloid scales, a single complete outer row of unicuspid teeth in both jaws and few additional inner buccal teeth, not forming a complete row. Most species described in the second half of the nineteenth century were synonymized with either *H. fasciatus* or *H. bimaculatus*. It took several decades until Burchard & Wickler (1965) recognized different forms within *H. fasciatus*. Finally, in 1979, Loiselle (1979) revised the genus *Hemichromis*. He described several new species (*H. frempongi*, *H. cristatus*, *H. lifalili*, *H. paynei*, *H. stellifer*) and removed others from synonymy (*H. elongatus* (Guichenot, 1861), *H. guttatus* Günther, 1862, *H. letourneuxi* Sauvage, 1880). Additionally, Loiselle transferred *Paratilapia cerasogaster* Boulenger, 1899 to the genus *Hemichromis*. In his taxonomic revision, Loiselle distinguished between two main species complexes, a *H. fasciatus* complex and a *H. bimaculatus* complex, of which the second, known as the 'jewel cichlids' among aquarists, has been subdivided into three species groups. Interestingly, though, Loiselle's work did not discuss the obvious differences in coloration and body size between the two species complexes. Species descriptions in Loiselle's revision have sometimes been ignored in later scientific publications because of difficulties to distinguish among the different species, especially when only preserved specimens were available (e. g., Leveque et al., 1992). Also in the aquaristic literature and studies focusing on the behavior of *Hemichromis*, erroneous species identifications based on body coloration are evident (e.g., Linke & Staeck, 2002).

Greenwood (1985a, b) published a detailed study on the anatomy of *Hemichromis*, with first notes on phyletic relationships in this genus. Even though he had examined several diagnostic characters, no explicit differentiation among species within as well as between the two complexes was made. Additionally, Greenwood mainly referred to only two species—*H. fasciatus* and *H. bimaculatus*—even though he mentioned all species sensu Loiselle (1979) and distinguished between the two groups based on their

vertebrae counts. Lamboj (2004) recognized two groups within the genus, mainly based on their color patterns and the maximum size of the species. He also discussed the problem of a high prevalence of incorrect species determination in the scientific literature and in aquaristic books and articles, and he indicated the possibility that the *H. bimaculatus* complex might be considered a genus of its own.

The most recent study (Bitja-Nyom et al., 2021) presented a systematic revision of the five-spotted *Hemichromis* species complex, based on an integrative approach combining morphometrics and meristics with mitochondrial DNA data. This study clarified some of the relationships among these species, suggesting synonymy of *H. frempongi* with *H. fasciatus* and describing a new species, *H. camerounensis*. Unfortunately, this study did not include samples from the complex' entire distribution range and covered only West Africa and parts of Lower Guinea.

Thus, the intrageneric diversity of the genus and the phylogenetic relationships among the species are still not entirely known. In the present study, we present a molecular phylogeny for the Hemichromini based on two mitochondrial and two nuclear markers. Together with data on morphology and body coloration, this is the basis for the first part of a formal revision of *Hemichromis*, with a revised diagnosis for this genus and the description of the new genus *Rubricatochromis* for the jewel cichlids. In addition, we confirm the validity of *H. angolensis* Steindachner 1865 and give a diagnosis and description for it, as it often was seen as species with uncertain status (e.g., Daget et al., 1991; Bitja-Nyom et al., 2021), even though currently considered as valid (Skelton, 2019).

Material and methods

DNA extraction, sequencing, and pre-processing of DNA sequences

The phylogenetic analyses are based on 65 samples collected by one of the authors (A.L.) between 1993 and 2016 under the respective permits (FD/PAO/9/V.6/337/93, Ministry of Agriculture, Fisheries department, Accra, Ghana; 003/11-7/MRST/D00/D20, Ministere de la Recherche Scientifique et Technique, Yaounde, Cameroon) or obtained from museum collections (16 species; Fig. 1, Suppl. Tab. 1). The fish were caught with hand nets or cast nets and euthanized with an overdose of MS222. Fin clips were immediately put in > 95% undenatured ethanol, voucher specimens were preserved in either 70% denatured alcohol or 10% formaldehyde, unless imported alive for qualitative characterization of body coloration. Extraction of whole genomic DNA from fin clips, was done using a Chelex 100 method (Walsh et al., 1991) or the Qiagen Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

PCR amplification and sequencing of parts of the mitochondrial 12S rRNA gene (12S, 603 bp, $N = 57$, primers: 12SA 5′-AAACTGGGATTAGATACCCCACTAT-3′, 12SB 5′-GAGGGTCACGGGCGGTGTGT-3′, Kocher et al., 1989), parts of the mitochondrial (cytochrome oxidase I gene (COI, 563 bp, $N = 47$, primers FishF1 5′-TCAACCAACCACAAAGACATTGGCAC-3′, FishR2 5′-ACTTCAGGGTGACCGAAGAATCAGAA-3′, Ward et al., 2005), parts of the nuclear recombination activation gene 1 (RAG1, 1080 bp, $N = 58$, primers: RAG1fF1 5′-CTGAGCTGCAGTCAGTACCATAAGATGT-3′, RAG1R3 5′-GTCTTGTGSAAGTAGTTGGT-3′, Lopez et al., 2004) and parts of the nuclear gene for the small ribosomal subunit protein ES7 (S7, 502 bp, $N = 64$, primers: S7RPEX1f 5′-TGGCCTCTTCCTTGGCCGTC-3′, S7RPEX2r 5′-AACTCGTCTGGCTTTTCGCC-3′, Chow & Hazama, 1998) was carried out at LGC Genomics GmbH (Berlin, Germany). All electropherograms were edited, trimmed, cleaned, and assembled into the final consensus sequences using BioEdit v5.0.9 (Hall, 1999). The same program was also used to check for frame-shifts and premature stop codons in the protein-coding gene sequences (*RAG1* and *CO1*). All sequences are deposited on GenBank under the accession numbers listed in Supplementary Table 1.

Phylogenetic analyses

Alignments were generated using MUSCLE (Edgar, 2004). Phylogenetic tree search based on single-locus alignments (we opted not to infer a tree based on the full concatenated data, because of quite some missing data for some of the samples), using *Anomalochromis thomasi* as outgroup (following Astudillo-Clavijo et al., 2022), employed maximum likelihood (ML) and Bayesian inference (BI) in IQ-TREE v1.6.11

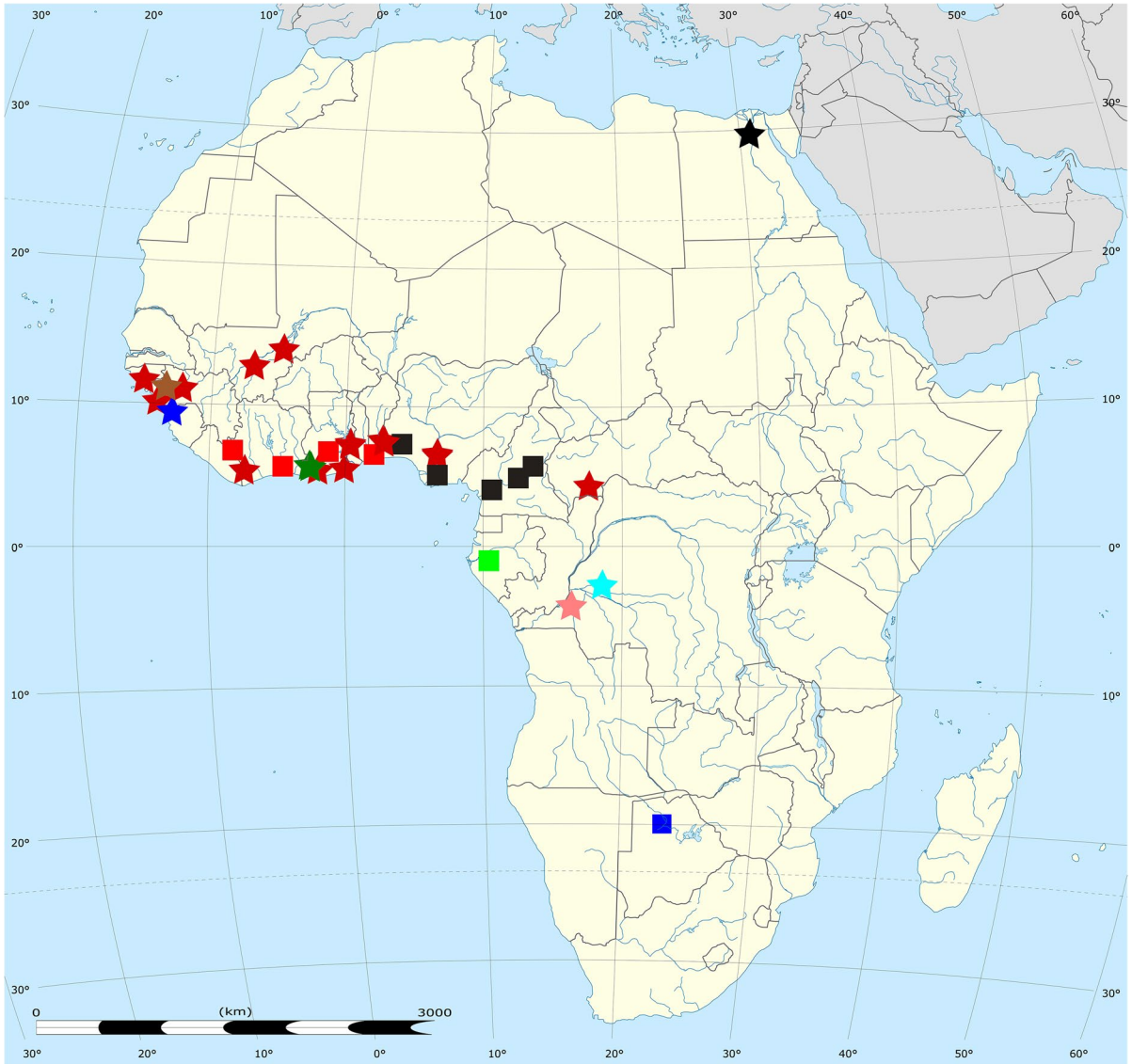


Fig. 1 Sampling sites for the samples (excl. aquarium imports) included in the molecular phylogenetic dataset. *Hemichromis* are indicated by squares (red, *H. fasciatus*; black, *H. camerounensis*; green, *H. elongatus*; blue, *H. angolensis*), *Rubricatochromis* gen. nov. by stars (black, *R. letourneuxi*; red, *R. guttatus*; dark blue, *R.* sp. 'Guinea 1'; brown, *R.* sp. 'Guinea 2'; green, *R.* cf. *paynei*; pink, *R. stellifer*; pale blue, *R. cerasogaster*. Background map © Eric Gaba – Wikimedia Commons user: Sting

(Nguyen et al., 2015) and MrBayes v3.2.6 (Ronquist et al., 2001), respectively. We used ModelFinder (Kalyaanamoorthy et al., 2017), implemented in IQ-TREE, to select the optimal model of sequence evolution based on the Bayesian Information Criterion (BIC; Supplementary File 1). We conducted a standard ML tree search, with nodal support assessed by means of standard nonparametric bootstrapping (500 replicates). BI tree searches were carried out for 20 million generations with a sampling frequency of 1000 generations, and the selected model of nucleotide substitution. The first 25% of trees were discarded as burn-in. Chain stationarity and parameter convergence (ESS > 200) were assessed in Tracer 1.7 (Rambaut et al., 2018). The post-burn-in trees were summarized in a 50% majority rule consensus tree.

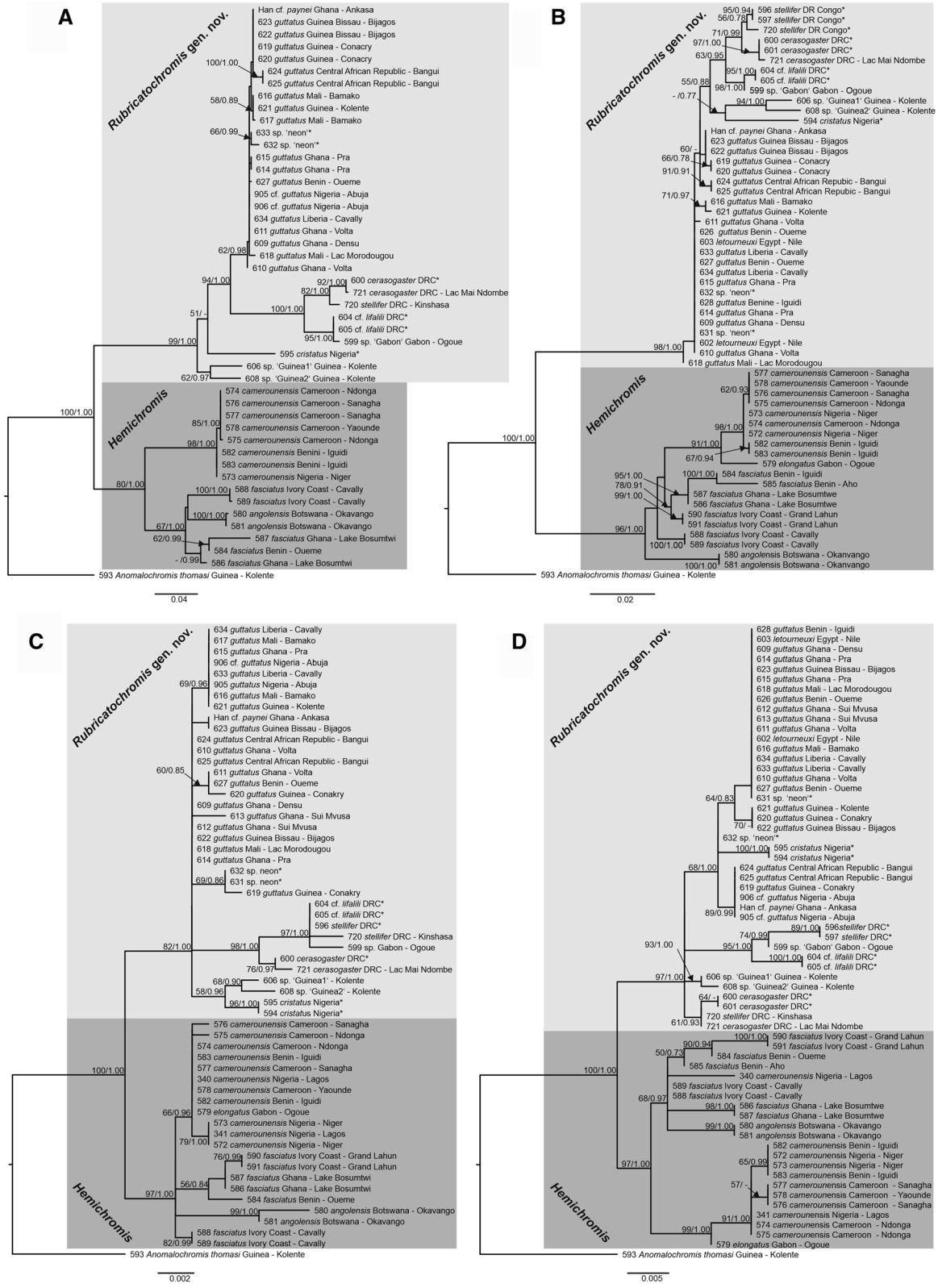
In addition, we inferred a chronogram with BEAST 2.5.2 (Bouckaert et al., 2019), employing a birth–death tree prior, a lognormal relaxed clock model and data partitioning by gene (but linking the two mitochondrial genes). Only samples for which at least three loci were available were included in the analysis. We ran three independent MCMC runs for 200 million generations and sampled model parameters every 1000 generation. LogCombiner (part of the BEAST2 package) was used to combine the three runs after discarding the first 25% of generations as burn-in. Stationarity and convergence of parameters were assessed in Tracer 1.7. Pooled post-burn-in effective sample size (ESS) was > 200 for all parameters. A maximum clade credibility tree was computed from the post-burn-in trees using TreeAnnotator (part of BEAST2 package) and visualized in FigTree 1.4.4 (available from http://tree.bio.ed.ac.uk/software/figtree/). Divergence times were calculated as mean node heights and the 95% highest posterior density (HPD) intervals. To calculate absolute divergence times, we assumed substitution rates of 1.0 and 2.0% per MY, a range typically observed in and applied for COI in fish (e.g., Lessios, 2008) and in line with the several times lower rate of COI as compared to the mitochondrial control region in cichlids (Genner et al., 2007; Koblmüller et al., 2009; Fischer et al., 2013). Substitution rates of the other markers were calculated relative to the COI rate in the course of the MCMC runs.

Morphological examination, life coloration, and behavioral observation

A total of 425 *Hemichromis* specimens of 16 species from various museum collections (Appendix 1—Supplementary Material) were used for morphological analyses. Twenty-two meristics and 25 morphometric measurements were taken following Barel et al. (1977): upper lateral-line scales, lower lateral-line scales, scale rows between upper and lower lateral line, total scales of lateral line, scales on caudal fin, scales around caudal peduncle, scale rows between origin of pectoral and pelvic fin, scale rows on cheek, scale rows on opercle, spines in dorsal fin, rays in dorsal fin, spines in anal fin, rays in anal fin, rays in pectoral fin, spines in pelvic fin, rays in pelvic fin, gill rakers on first ceratobranchial, gill rakers on first epibranchial, gill rakers on first pharyngobranchial, gill rakers on first outer branch, tooth rows in upper jaw, tooth rows in lower jaw, standard length, body depth, head length, head depth, snout length, eye diameter, postorbital length, mouth width, interorbital width, length of dentigerous arm of premaxilla, lower jaw length, cheek depth, preorbital distance, caudal peduncle length, caudal peduncle depth, predorsal length, preanal length, prepectoral length, prepelvic length, dorsal fin base, anal fin base, longest dorsal fin ray, longest anal fin ray, longest pectoral fin ray, longest pelvic fin ray. All measurements were taken on the left side with digital calipers with an accuracy of ± 0.03 mm. The clearing and staining protocol to examine bones and cartilage and taking vertebrae counts followed Dingerkus & Uhler (1977).

To visualize variation in morphospace among individuals and groups, principal component analyses (PCA) were conducted in PAST 4.03 (Hammer et al., 2001) on the correlation matrix of the meristics and, for the measurements, the covariance matrix of the residuals from the regression of log-transformed measurements on log-transformed SL (to avoid allometric effects; Klingenberg, 2016). Morphological variation was studied on the four main PCs that describe variation in meristics and morphometrics, respectively. Separate PCAs were done on (i) the full morphometric, (ii) the full meristic, (iii) a five-spotted *Hemichromis* morphometric, and (iv) a five-spotted *Hemichromis* meristic dataset. Invariant characters in the five-spotted *Hemichromis* meristic dataset (i.e., number of spines in pelvic fin) were removed, as were individuals with missing data for any count or measurement. We used PERMANOVAs (9999 permutations) to assess whether five-spotted *Hemichromis* and jewels cichlids, and species within the five-spotted *Hemichromis* complex were significantly separated in morphospace. We did not further analyze the jewel cichlids as a more detailed study on this complex is underway. Separate PERMANOVAs were run on all the PCs derived from the different morphometric and meristic datasets. In addition, linear discriminant analyses (LDA) on the morphometric and meristic datasets, conducted in PAST, were used to test the morphological diagnosability of genetically distinct groups. The classification accuracy of the LDA was assessed through a jackknife approach ('leave-one-out' cross-validation).

Possible diagnostic traits were identified by pairwise inter-group comparisons of size corrected (%SL or %HL) measurements and of the raw data of the


A
Rubricatochromis gen. nov.
Hemichromis
Han cf. paynei Ghana - Ankasa
623 guttatus Guinea Bissau - Bijagos
622 guttatus Guinea Bissau - Bijagos
619 guttatus Guinea - Conacry
620 guttatus Guinea - Conacry
624 guttatus Central African Republic - Bangui
625 guttatus Central African Republic - Bangui
616 guttatus Mali - Bamako
621 guttatus Guinea - Kolente
617 guttatus Mali - Bamako
633 sp. 'neon'*
632 sp. 'neon'*
615 guttatus Ghana - Pra
614 guttatus Ghana - Pra
627 guttatus Benin - Oueme
905 cf. guttatus Nigeria - Abuja
906 cf. guttatus Nigeria - Abuja
634 guttatus Liberia - Cavally
611 guttatus Ghana - Volta
609 guttatus Ghana - Densu
618 guttatus Mali - Lac Morodougou
610 guttatus Ghana - Volta
600 cerasogaster DRC*
721 cerasogaster DRC - Lac Mai Ndombe
720 stellifer DRC - Kinshasa
604 cf. lifalili DRC*
605 cf. lifalili DRC*
599 sp. 'Gabon' Gabon - Ogoue
595 cristatus Nigeria*
606 sp. 'Guinea1' Guinea - Kolente
608 sp. 'Guinea2' Guinea - Kolente
574 camerounensis Cameroon - Ndonga
576 camerounensis Cameroon - Sanagha
577 camerounensis Cameroon - Sanagha
578 camerounensis Cameroon - Yaounde
575 camerounensis Cameroon - Ndonga
582 camerounensis Benini - Iguidi
583 camerounensis Benini - Iguidi
573 camerounensis Nigeria - Niger
588 fasciatus Ivory Coast - Cavally
589 fasciatus Ivory Coast - Cavally
580 angolensis Botswana - Okavango
581 angolensis Botswana - Okavango
587 fasciatus Ghana - Lake Bosumtwi
584 fasciatus Benin - Oueme
586 fasciatus Ghana - Lake Bosumtwi
593 Anomalochromis thomasi Guinea - Kolente
0.04
B
Rubricatochromis gen. nov.
Hemichromis
596 stellifer DR Congo*
597 stellifer DR Congo*
720 stellifer DR Congo*
600 cerasogaster DRC*
601 cerasogaster DRC*
721 cerasogaster DRC - Lac Mai Ndombe
604 cf. lifalili DRC*
605 cf. lifalili DRC*
599 sp. 'Gabon' Gabon - Ogoue
606 sp. 'Guinea1' Guinea - Kolente
608 sp. 'Guinea2' Guinea - Kolente
594 cristatus Nigeria*
Han cf. paynei Ghana - Ankasa
623 guttatus Guinea Bissau - Bijagos
622 guttatus Guinea Bissau - Bijagos
619 guttatus Guinea - Conacry
620 guttatus Guinea - Conacry
624 guttatus Central African Repubic - Bangui
625 guttatus Central African Repubic - Bangui
616 guttatus Mali - Bamako
621 guttatus Guinea - Kolente
611 guttatus Ghana - Volta
626 guttatus Benin - Oueme
603 letourneuxi Egypt - Nile
633 guttatus Liberia - Cavally
627 guttatus Benin - Oueme
634 guttatus Liberia - Cavally
615 guttatus Ghana - Pra
632 sp. 'neon'*
628 guttatus Benine - Iguidi
614 guttatus Ghana - Pra
609 guttatus Ghana - Densu
631 sp. 'neon'*
602 letourneuxi Egypt - Nile
610 guttatus Ghana - Volta
618 guttatus Mali - Lac Morodougou
577 camerounensis Cameroon - Sanagha
578 camerounensis Cameroon - Yaounde
576 camerounensis Cameroon - Sanagha
575 camerounensis Cameroon - Ndonga
573 camerounensis Nigeria - Niger
574 camerounensis Cameroon - Ndonga
572 camerounensis Nigeria - Niger
582 camerounensis Benin - Iguidi
583 camerounensis Benin - Iguidi
579 elongatus Gabon - Ogoue
584 fasciatus Benin - Iguidi
585 fasciatus Benin - Aho
587 fasciatus Ghana - Lake Bosumtwe
586 fasciatus Ghana - Lake Bosumtwe
590 fasciatus Ivory Coast - Grand Lahun
591 fasciatus Ivory Coast - Grand Lahun
588 fasciatus Ivory Coast - Cavally
589 fasciatus Ivory Coast - Cavally
580 angolensis Botswana - Okanvango
581 angolensis Botswana - Okanvango
593 Anomalochromis thomasi Guinea - Kolente
0.02
C
Rubricatochromis gen. nov.
Hemichromis
634 guttatus Liberia - Cavally
617 guttatus Mali - Bamako
615 guttatus Ghana - Pra
906 cf. guttatus Nigeria - Abuja
633 guttatus Liberia - Cavally
905 guttatus Nigeria - Abuja
616 guttatus Mali - Bamako
621 guttatus Guinea - Kolente
Han cf. paynei Ghana - Ankasa
623 guttatus Guinea Bissau - Bijagos
624 guttatus Central African Republic - Bangui
610 guttatus Ghana - Volta
625 guttatus Central African Republic - Bangui
611 guttatus Ghana - Volta
627 guttatus Benin - Oueme
620 guttatus Guinea - Conakry
609 guttatus Ghana - Densu
613 guttatus Ghana - Sui Mvusa
612 guttatus Ghana - Sui Mvusa
622 guttatus Guinea Bissau - Bijagos
618 guttatus Mali - Lac Morodougou
614 guttatus Ghana - Pra
632 sp. neon*
631 sp. neon*
619 guttatus Guinea - Conakry
604 cf. lifalili DRC*
605 cf. lifalili DRC*
596 stellifer DRC*
720 stellifer DRC - Kinshasa
599 sp. Gabon - Ogoue
600 cerasogaster DRC*
721 cerasogaster DRC - Lac Mai Ndombe
606 sp. 'Guinea1' - Kolente
608 sp. 'Guinea2' - Kolente
595 cristatus Nigeria*
594 cristatus Nigeria*
576 camerounensis Cameroon - Sanagha
575 camerounensis Cameroon - Ndonga
574 camerounensis Cameroon - Ndonga
583 camerounensis Benin - Iguidi
577 camerounensis Cameroon - Sanagha
340 camerounensis Nigeria - Lagos
578 camerounensis Cameroon - Yaounde
582 camerounensis Benin - Iguidi
579 elongatus Gabon - Ogoue
573 camerounensis Nigeria - Niger
341 camerounensis Nigeria - Lagos
572 camerounensis Nigeria - Niger
590 fasciatus Ivory Coast - Grand Lahun
591 fasciatus Ivory Coast - Grand Lahun
587 fasciatus Ghana - Lake Bosumtwi
586 fasciatus Ghana - Lake Bosumtwi
584 fasciatus Benin - Oueme
580 angolensis Botswana - Okavango
581 angolensis Botswana - Okavango
588 fasciatus Ivory Coast - Cavally
589 fasciatus Ivory Coast - Cavally
593 Anomalochromis thomasi Guinea - Kolente
0.002
D
Rubricatochromis gen. nov.
Hemichromis
628 guttatus Benin - Iguidi
603 letourneuxi Egypt - Nile
609 guttatus Ghana - Densu
614 guttatus Ghana - Pra
623 guttatus Guinea Bissau - Bijagos
615 guttatus Ghana - Pra
618 guttatus Mali - Lac Morodougou
626 guttatus Benin - Oueme
612 guttatus Ghana - Sui Mvusa
613 guttatus Ghana - Sui Mvusa
611 guttatus Ghana - Volta
602 letourneuxi Egypt - Nile
616 guttatus Mali - Bamako
634 guttatus Liberia - Cavally
633 guttatus Liberia - Cavally
610 guttatus Ghana - Volta
627 guttatus Benin - Oueme
631 sp. 'neon'*
621 guttatus Guinea - Kolente
620 guttatus Guinea - Conakry
622 guttatus Guinea Bissau - Bijagos
632 sp. 'neon'*
595 cristatus Nigeria*
594 cristatus Nigeria*
624 guttatus Central African Republic - Bangui
625 guttatus Central African Republic - Bangui
619 guttatus Guinea - Conakry
906 cf. guttatus Nigeria - Abuja
Han cf. paynei Ghana - Ankasa
905 cf. guttatus Nigeria - Abuja
596stellifer DRC*
597 stellifer DRC*
599 sp. 'Gabon' Gabon - Ogoue
604 cf. lifalili DRC*
605 cf. lifalili DRC*
606 sp. 'Guinea1' Guinea - Kolente
608 sp. 'Guinea2' Guinea - Kolente
600 cerasogaster DRC*
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720 stellifer DRC - Kinshasa
721 cerasogaster DRC - Lac Mai Ndombe
590 fasciatus Ivory Coast - Grand Lahun
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584 fasciatus Benin - Oueme
585 fasciatus Benin - Aho
340 camerounensis Nigeria - Lagos
589 fasciatus Ivory Coast - Cavally
588 fasciatus Ivory Coast - Cavally
586 fasciatus Ghana - Lake Bosumtwe
587 fasciatus Ghana - Lake Bosumtwe
580 angolensis Botswana - Okavango
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577 camerounensis Cameroon - Sanagha
578 camerounensis Cameroon - Yaounde
576 camerounensis Cameroon - Sanagha
341 camerounensis Nigeria - Lagos
574 camerounensis Cameroon - Ndonga
575 camerounensis Cameroon - Ndonga
579 elongatus Gabon - Ogoue
593 Anomalochromis thomasi Guinea - Kolente
0.005

◂**Fig. 2** Maximum likelihood (ML) trees showing the phylogenetic relationships within the Hemichromini based on single-locus data: **a** COI, **b** 12S, **c** RAG1, and **d** S7. As measures of nodal support bootstrap support values (for ML; only values > 50 are shown) and posterior probabilities (from Bayesian Inference; only values > 0.7 are shown) are depicted

counts by Mann–Whitney *U* tests with sequential Bonferroni correction (Rice, 1989).

Coloration

In addition, live specimens collected by one of the authors (A.L) or imported for the ornamental fish trade were used for qualitatively assessing live body coloration. With the exception of specimens of *H.* sp. 'neon' and *H.* cf. *lifalili* none of these specimens was included in the genetic analysis. In addition, none of these specimens was included in the morphological analyses, as morphometric data might be strongly affected by aquarium conditions (e.g., Kerschbaumer et al., 2011).

Results

Molecular phylogeny

As phylogenetic inference based on ML and BI revealed highly consistent tree topologies, only the ML trees are shown in Fig. 2. All single gene analyses yielded highly congruent results, with differences in branching patterns mainly concerning poorly supported nodes. Consistently, two deeply divergent clades were found, one containing the five-spotted *Hemichromis* species and the other comprising the so-called jewel cichlids. Within the five-spotted *Hemichromis*, the clustering does not correspond to accepted species boundaries. Whereas *H. elongatus* from Gabon and *H. camerounensis* form a distinct clade (with the exception of one Nigerian individual in the S7 tree), the *H. elongatus*-like (based on coloration) individuals from Botswana are quite divergent from these two species and more closely related to *H. fasciatus*. Consistent with Bitja-Nyom et al. (2021), fish previously regarded as a distinct species, *H. frempongi* (from Lake Bosomtwe, Ghana; samples 586 & 587), are placed within the *H. fasciatus* clade. Within the jewel cichlids, most West and North African samples (*H. guttatus*, *H. paynei*, *H. letourneuxi*, *H.* sp. 'neon') are very homogeneous with only low levels of divergence and haplotype sharing even over large geographic scales. The only exceptions are the two undescribed species from Guinea, that are clearly distinct from the other West African samples, and *H. cristatus*, which also constitutes a separate clade. The Central African species (*H. cerasogaster*, *H. lifalili*, *H. stellifer, H. sp.* '*Gabon*') show higher levels of interspecific divergence, even though some haplotype sharing also occurs among these species.

The general patterns observed in the single gene trees are corroborated by the time-calibrated tree based on the concatenated dataset. The five-spotted *Hemichromis* and the jewel cichlids were estimated to have diverged 5.89 (95%HPD 3.67–8.81) to 11.79 (95%HPD 7.35–17.61) million years ago (mya) (Fig. 3). The most recent common ancestors (MRCA) of the five-spotted *Hemichromis* and the jewel cichlids were estimated at 2.71 (95%HPD 1.63–4.00) to 5.41 (95%HPD 3.26–8.01) mya and 2.61 (95%HPD 1.71–3.82) to 5.22 (95%HPD 3.42–7.65) mya, respectively. With inferred divergence times of 1.84 (95%HPD 1.11–2.77) to 3.69 (95%HPD 2.21–5.55) and 1.58 (95%HPD 0.93–2.42) to 3.16 (95%HPD 1.85–4.84), *H. fasciatus* from the Cavally River (Ivory Coast) and the *H. elongatus*-like fish from Botswana appear to be quite divergent from the remainder of this clade. Within the *H. elongatus* clade, comprising *H. elongatus* from the Ogooue River in Gabon, the species' type locality, and *H. camerounensis*, the two species diverged about 0.94 (95%HPD 0.41–1.71) to 1.87 (95%HPD 0.81–3.43) mya. The MRCA of *H. camerounensis* was dated to only 0.22 (95%HPD 0.09–0.40) to 0.43 (95%HPD 0.19–0.79) mya. Within the jewel cichlids, the first series of cladogenetic events between 2.61 (95%HPD 1.71–3.82) to 5.22 (95%HPD 3.42–7.65) and 1.83 (95%HPD 1.08–2.72) to 3.65 (95%HPD 2.16–5.44) mya gave rise to four major clades, the first comprising the two undescribed species from Guinea, the second *H. cerasogaster*, *H. lifalili*, *H.* sp. Gabon and *H. stellifer* from Central Africa, the third containing *H. cristatus* from Nigeria, and the fourth including *H. guttatus* and *H. paynei* from Nigeria to Guinea. With an MRCA dated to 0.49 (95%HPD 0.28–0.81) to 0.99 (95%HPD 0.56–1.61) mya, intra-clade divergence is comparatively recent in the fourth jewel cichlid clade.

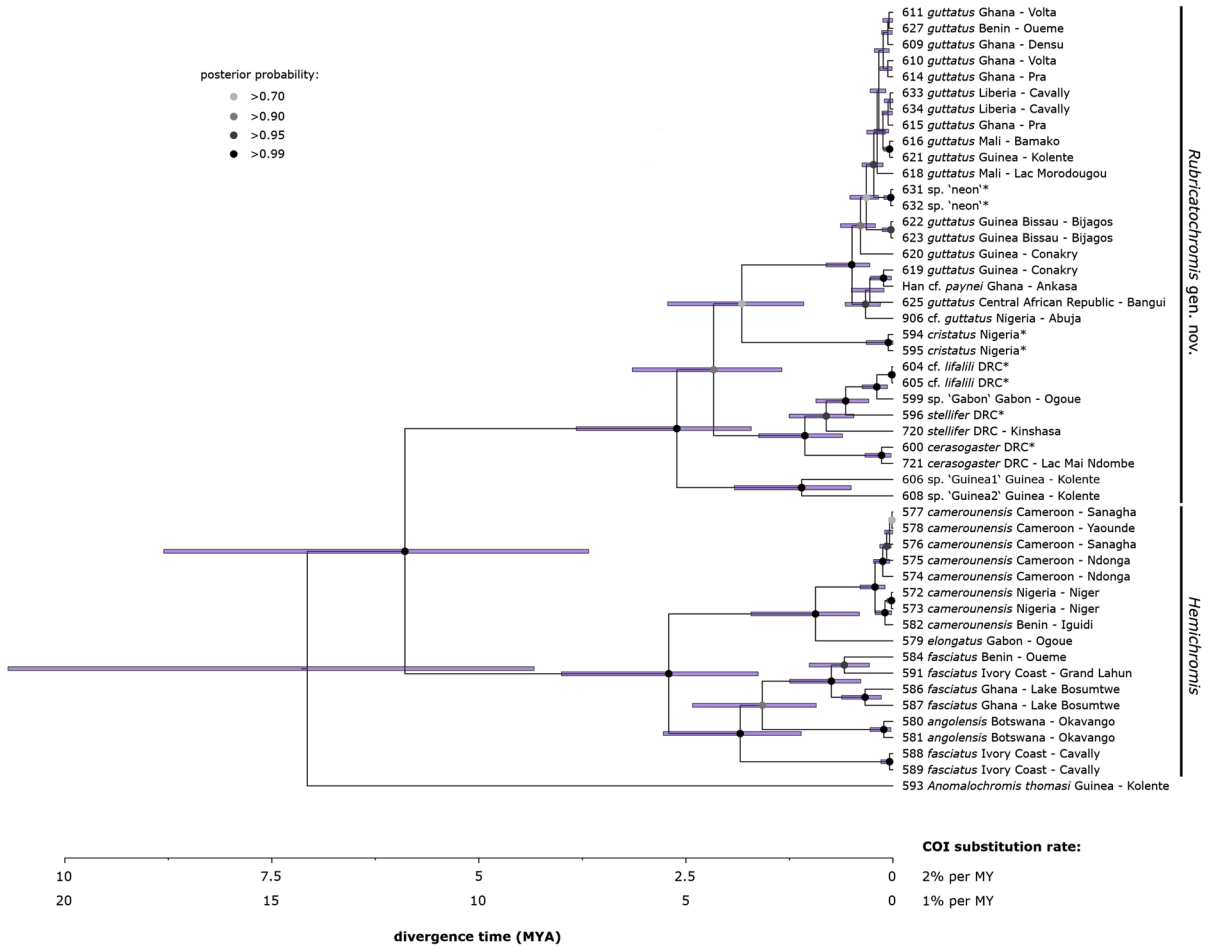

Fig. 3 Dated phylogeny inferred using BEAST, based on the concatenated dataset, with divergence times calculated assuming a maximum and minimum substitution rate of 2.0% (above) and 1.0% (below) per MY for COI, respectively. Posterior probabilities are only shown for nodes with support > 0.7. Only samples for which data of at least three loci were available were included in the analysis

Morphology—full dataset

Both the morphometric and the meristic PCAs based on the full dataset separated, albeit with some overlap, the jewel cichlids from the five-spotted *Hemichromis* (Figs. 4, 5). In the morphometric PCA, the first four PCs explained 69.4% of the total variation (Fig. 4—Scree plot). PC1, which separates the two main groups, explains 38.5% of the variation and is positively loaded with high values for body and caudal peduncle depth, length of dorsal and anal fin and length of the longest fin rays, and negatively loaded with characters related to snout length, mouth size and caudal peduncle length (Suppl. Tab. 2). PC2 and PC3, or any of the additional PCs, did not provide any further resolution for separating the jewel cichlids from the five-spotted *Hemichromis*. Despite the overlap, PERMANOVA showed a significant difference between the two groups ($F = 52.51$; $P < 0.0001$). The LDA based on the morphometric data separates most specimens according to their prior classification (i.e., specimen attribution to each of the two main lineages). Classification accuracy based on successive specimen deletion (jackknife) is high (correct classification rate = 95.78%).

In the meristic PCA, the first four PCs explained 50.6% of the total variation (Fig. 5—Scree plot). PC1, separating the two main groups without an overlap,

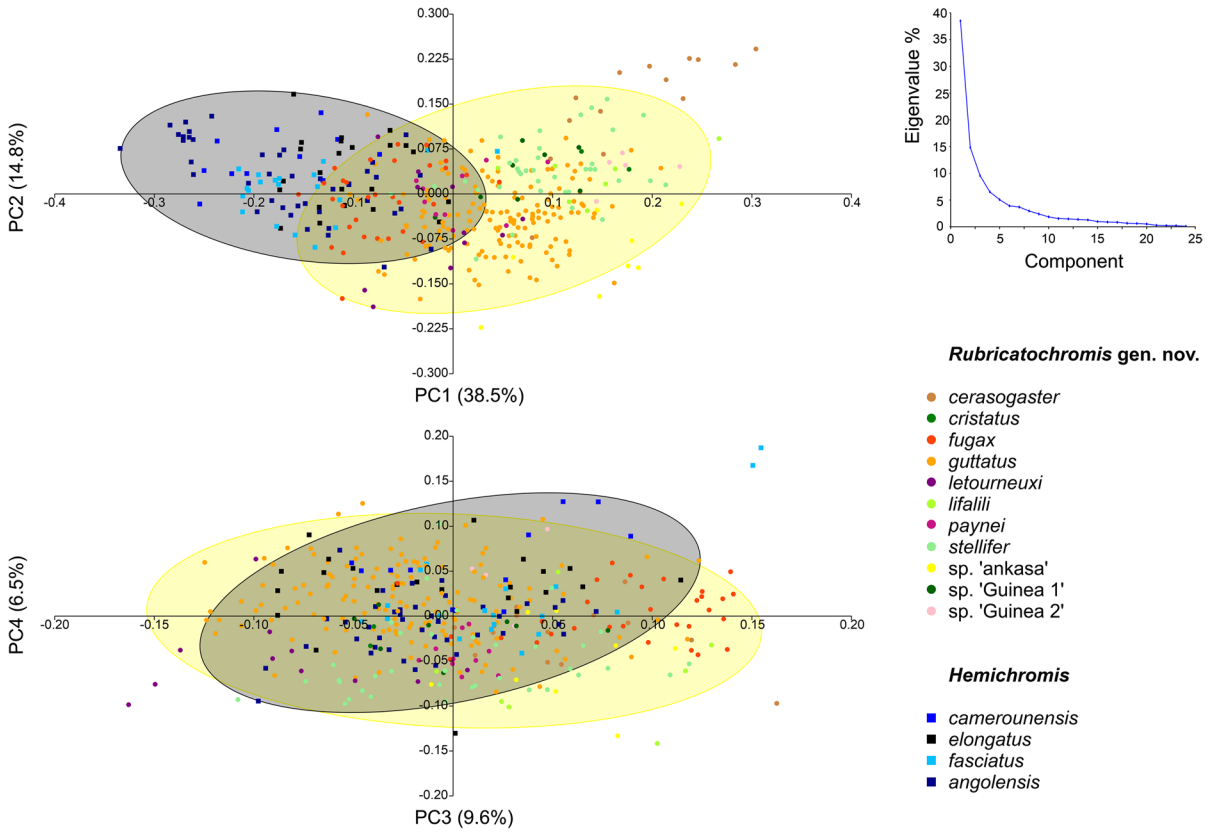

Fig. 4 Scatterplots of the scores on the principal components based on the residuals from the regression of log-transformed measurements on log-transformed standard length of the entire dataset. 95% confidence ellipses are shown for the two genera *Hemichromis* (black) and *Rubricatochromis* gen. nov. (yellow). The Scree plot (small inset to the right) shows how much of the total variation is explained by the different components

explains 24.9% of the variation with highest loadings from (lower) lateral-line scales, scale rows on cheek, rays in anal and dorsal fin, and the various of gill raker counts (Suppl. Tab. 3). PC2 and PC3, or any of the additional PCs, did not provide any further resolution for separating the jewel cichlids from the five-spotted *Hemichromis*. PERMANOVA showed a significant difference between the two groups ($F = 97.52$; $P < 0.0001$). Likewise, the LDA based on the meristic variables almost perfectly separates specimens according to their prior classification, with a jackknifed classification accuracy of 99.46%.

Morphology—five-spotted *Hemichromis*

In the morphometric PCA of the five-spotted *Hemichromis* complex, the first four PCs explained 63.9% of the total variation (Fig. 6—Scree plot). Despite a large overlap in the PCA plot (Fig. 6; for loadings see Suppl. Tab. 4), the global PERMANOVA was significant ($F = 2.869$, $P < 0.0001$), as were all pairwise post hoc tests (all P values < 0.01; Suppl. Tab. 4). In the morphometric LDA, the jackknifed classification accuracy was 71.88%, thus indicating a fair number of misclassified specimens.

In the PCA based on the meristic data, the first four PCs explained 44.9% of the total variation (Fig. 7—Scree plot). Again, a broadly overlapping morphospace among species became evident in the PCA plot (Fig. 7; for loadings see Suppl. Table 5). Nonetheless, the global PERMANOVA was significant ($F = 3.661$, $P < 0.0001$), as were all pairwise post hoc tests (all P values < 0.05; Suppl. Tab. 5). In the meristic LDA, classification accuracy was 82.22%.

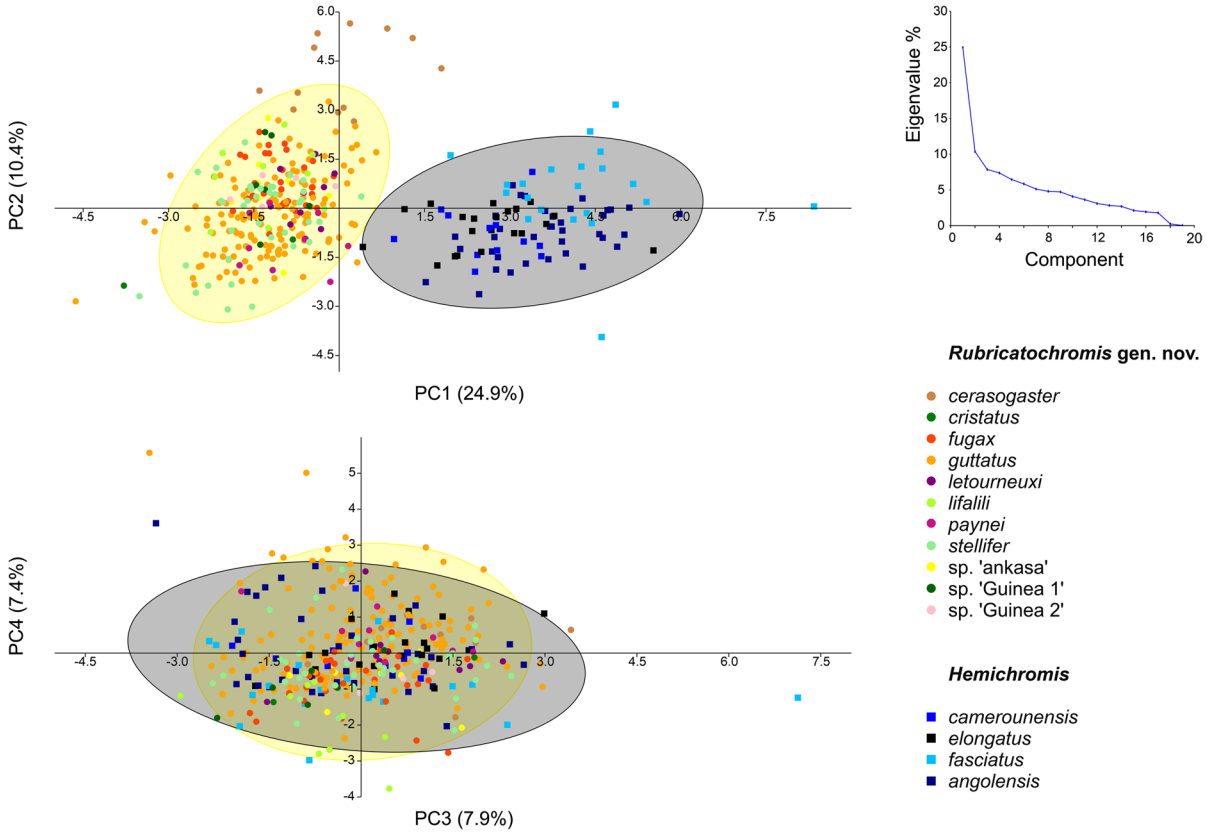

Fig. 5 Scatterplots of the scores on the principal components based on the meristics of the entire datasets. 95% confidence ellipses are shown for the two genera *Hemichromis* (black) and *Rubricatochromis* gen. nov. (yellow). The Scree plot (small inset to the right) shows how much of the total variation is explained by the different components

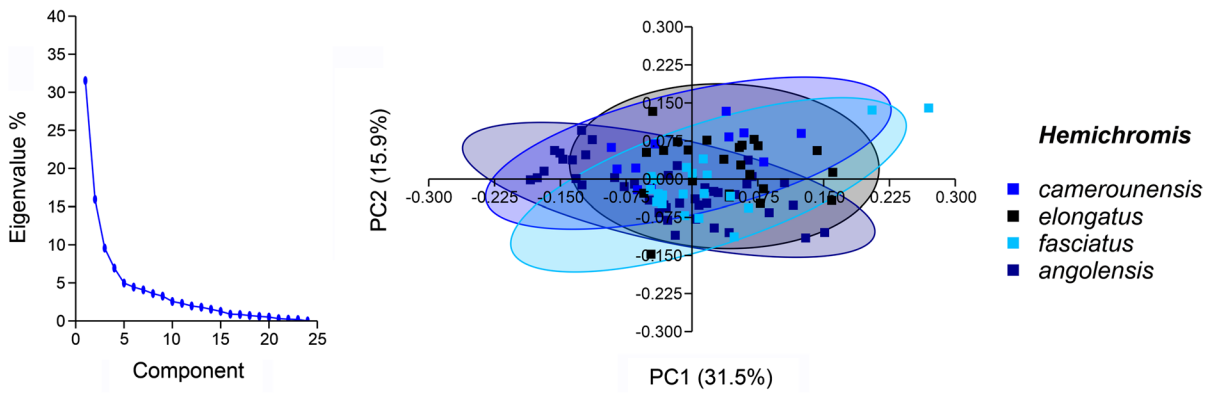

Fig. 6 Scatterplots of the scores on the principal components based on the residuals from the regression of log-transformed measurements on log-transformed standard length of *Hemichromis* datasets. 95% confidence ellipses are shown for the four species of *Hemichromis* (light blue, *H. fasciatus*; blue, *H. camerounensis*; dark blue, *H. angolensis*; black, *H. elongatus*). The Scree plot shows how much of the total variation is explained by the different components

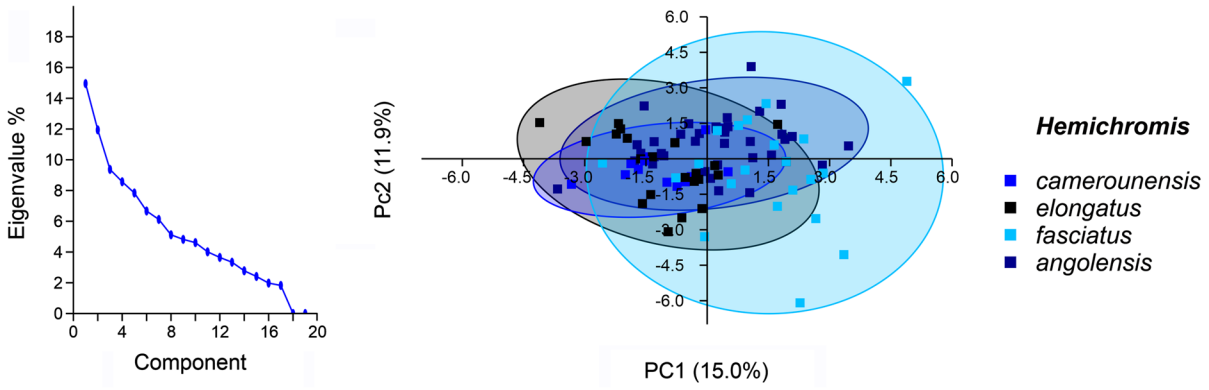

Fig. 7 Scatterplots of the scores on the principal components based on the meristics of the *Hemichromis* dataset. 95% confidence ellipses are shown for the four species of *Hemichromis* (light blue, *H. fasciatus*; blue, *H. camerounensis*; dark blue, *H. angolensis*; black, *H. elongatus*). The Scree plot shows how much of the total variation is explained by the different components

Coloration

Adult body color patterns (five-spotted *Hemichromis* vs. jewel cichlids) also support the two groups. In the five-spotted *Hemichromis*, adult specimens always exhibit a more yellow body coloration and 5 black spots (one on the outer edge of the opercle, four on the body side), while in the jewel cichlids, a maximum of three black spots (one on the outer edge of the opercle, the second on flanks, the third one on caudal peduncle) may be visible, of which the second and/or the third can disappear in adults specimens, depending on the species (compare Fig. 9 with 12).

Based on the molecular phylogenetic trees, morphological data, and coloration patterns in adult specimens, we herein recognize two diagnosably distinct genera and provide a formal description of a new genus for the jewel cichlids.

Diagnosis for genera:

Hemichromis Peters 1858

Type species: *Hemichromis fasciatus* Peters 1858 (Fig. 8).

Diagnosis

Medium-sized to large cichlids (93.7–187.4 mm max. SL), of ovoid body shape with big head and highly protruding mouth. Teeth in both jaws unicuspid, usually situated in one outer row; unregular inner buccal teeth variably disposed possible in some, mainly bigger specimens. Scales cycloid. 16 scales around caudal peduncle. Vertebrae counts 26–28, with 14–15 abdominal and 12–15 caudal. Infraorbital series containing a lachrymal with four openings of the laterosensory system and five additional tubular bones. Microbranchiospines absent

Fig. 8 Lectotype of *Hemichromis* fasciatus (ZMB 2811), SL 116.7 mm. Scale bar = 10 mm

Fig. 9 Comparison of standard coloration of specimens of *Hemichromis* from different sampling sites. **A** *H. camerounensis,* Benin, Iguidi River (Niger River system), coll. A.L.; **B** *H. camerounensis,* Nigeria, Niger River system (trade); **C** *H. camerounensis,* Cameroon, Mungo River system, coll. A.L.; **D** *H. elongatus,* Gabon, Ogooue River system, coll. A.L.; **E** *H. angolensis,* Botswana, Okavango Delta; **F** *H. angolensis,* Angola, Cuanza River. Photographs and coll. for **E** and **F** Roger Bills, SAIAB. Size of specimens was not measured

on gill arch 1, but present on outer aspects of gill arches 2 to 4, rarely on both aspects of certain of these arches. Caudal skeleton with well-developed hypurapophysis on parhypural. Upper lateral line clearly separated from dorsal fin base. Sexual dichromatism and dimorphism poorly developed or absent. Semiadults and adults of all included species with usually five distinct black spots visible in most behavioral situations, of which the first is on opercle, the last on end of caudal peduncle. Single specimens may show one more or less spot on one of the body sides.

Differs from other hemichromines in greater maximum SL (49.8 mm for *Anomalochromis*, 36.9–85.2 mm for species of *Rubricatochromis* vs. 93.7–187.4 mm for species of *Hemichromis*) and in a unique coloration pattern of four to five dark to black spots on body sides (including opercular spot) vs. a maximum of three in the two other genera. Adults of *Hemichromis* exhibit a black band visible in most behavioral situations (absent or just poorly visible in situations with highest stress), running from the forehead through the eye to the angle of the mouth, which is not present in the other two genera. Mouth highly protrudable, vs. moderately in *Rubricatochromis* and poorly in *Anomalochromis*.

For a more detailed description of coloration and anatomy of single species, see Loiselle (1979), Greenwood (1985a, b), and Bitja-Nyom et al. (2021). In general, we confirm the information about differences among species as given in Bitja-Nyom et al. (2021), with the only exception that the pattern of two red spots, flanking opposite sides of the large black opercular spot as given to be typical for *H. camerounensis,* separating this species from *H. elongatus,* has been found in our study in part of the samples of *H. elongatus* and *H. angolensis* (Fig. 9). Therefore, we regard this character as non-diagnostic.

All species form pairs and are free substrate spawners with intense broodcare, as described in Loiselle (1979) and Lamboj (2004).

Included species:

Hemichromis fasciatus Peters, 1858

Hemichromis elongatus (Guichenot, 1861)

Hemichromis camerounensis Bitja-Nyom, Agnése, Pariselle, Bilong-Bilong & Snoeks, 2021

Hemichromis angolensis, Steindachner, 1865

Distribution: In both forest and savannah biotopes along the West African coast from Senegal to Angola, also in the Nile basin and from Lake Chad to Ituri River in the Congo River basin and Upper Zambezi.

For *Hemichromis angolensis* Steindachner, 1865 we provide a new diagnosis and redescription, based on a neotype (Fig. 10)—as the holotype is lost (Bell-Cross, 1975)—and 44 additional specimens designated as paratypes. The neotype, collected by Schoenfeldt in 1957, has been chosen as the original type had the rather vague information 'Angola' as type locality, but according to Bitja-Nyom et al. (2021), the Cuanza basin seems to be the most probable location, and this specimen was also collected in the Cuanza basin in Angola. Additionally, the data given for the original holotype in the original description are fitting well with the data for the new type series.

Diagnosis: *H. angolensis* can be distinguished from *H. fasciatus* by the absence of small black dots between the first three dark stripes on the flanks of the body of adults in most populations of *H. fasciatus*. It can further be distinguished from *H. fasciatus* by a combination of overlapping morphometrics, mainly by a high number of anal fin soft rays [9–11 (median 10) vs 8–10 (9)]; a greater body depth [30.3–42.4 (mean 35.8) vs 27.1–37.8 (mean 34.2) % SL]; a greater head depth [51.4–70.6 (mean 61.5) vs 48.4–66.0 (mean 56.2) % HL]; shorter length of lower jaw [(35.4–45.7 (mean 42.6) vs 40.0–49.0 (mean 43.4) % HL].

H. angolensis can be distinguished from *H. camerounensis* by a combination of overlapping morphometrics, mainly by a greater prepelvic distance [39.0–52.0 (mean 42.1) vs 37.6–48.5 (mean 42.8) % SL]; a shorter length of anal fin base [13.2–17.7 (mean 15.8) vs 14.5–18.3 (mean 16.5) % SL]; greater preorbital distance [(10.3–18.1 (mean 14.0) vs 9.1–13.2 (mean 10.8) % HL].

H. angolensis can be distinguished from *H. elongatus* by a combination of overlapping morphometrics, mainly by a shorter head length [33.6–39.7 (mean 36.8) vs 36.9–40.2 (mean 38.1) % SL]; a shorter length of anal fin base [13.2–17.7 (mean 15.8) vs 14.8–22.8 (mean 16.7) % SL]; greater eye orbit diameter [(19.8–31.0 (mean 24.2) vs 18.8–28.8 (mean 23.0) % HL]; shorter length of lower jaw [(35.4–45.7 (mean 42.6) vs 38.9–46.2 (mean 43.6) % HL].

The distributions of the four species do not overlap. *H. fasciatus* is distributed in West Africa, *H. camerounensis* occurs (probably) from the most-eastern parts of Benin (tributaries of the Niger River, e.g., Iguidi River A.L. pers. coll.) through Nigeria to Cameroon, Lobe River system, *H. elongatus* from Cameroon—Ntem, and Dja River basins—through Gabon and the Congo River system, while *H. angolensis* occurs in southern African river systems from the Cuanza region to the Zambezi River system, including the Kafue system and Okavango floodplains.

Redescription: Measurements and meristics for neotype and 44 paratypes in Table 1. Maximum observed size: 134.5 mm SL. No sex-specific morphological differentiation. General body shape deep and moderately elongated; dorsal and anal fins reaching or exceeding level of caudal fin origin; dorsal fin straight with a high number of soft rays, soft rays 5 to 6 longest. Length of pectoral and pelvic fin rays decreasing from first to last branched rays. Caudal fin moderately truncate or rounded; caudal peduncle from deeper than long to longer than deep; cycloid

Fig. 10 Neotype of *Hemichromis angolensis*, ZSM 22145, SL 109.3 mm. Photograph by ZSM, scale bar = 10 mm

Table 1 Morphometric and meristic data of the neotype and neotype + 44 paratypes of *Hemichromis angolensis*

	Neotype	Mean	SD	Range
Standard length mm	109.32	41.4		30.0–58.3
Body depth	36.0	35.3	2.8	30.3–42.4
Head length	36.0	36.8	1.6	33.6–39.7
Caudal peduncle length	13.4	13.0	0.8	11.5–14.9
Caudal peduncle depth	14.3	14.1	0.8	12.7–16.1
Dorsal fin base	54.2	51.6	3.0	45.4–56.8
Anal fin base	17.0	15.8	1.1	13.2–17.7
Predorsal distance	32.9	34.6	2.1	30.5–38.4
Preanal distance	70.5	72.8	1.5	70.2–77.0
Prepectoral distance	37.2	38.5	2.4	35.1–46.9
Prepelvic distance	41.7	42.1	2.3	39.0–52.0
Longest dorsal fin ray	18.4	16.9	2.2	12.1–22.3
Longest anal fin ray	19.6	17.4	1.3	14.8–20.3
Longest pectoral fin ray	20.7	18.5	1.0	16.6–21.1
Longest pelvic fin ray	25.3	24.9	2.6	20.1–32.4
% HL				
Head depth	58.6	61.5	5.4	51.4–70.6
Snout length	34.7	33.7	2.7	27.1–40.0
Eye diameter	22.3	24.2	3.2	19.8–31.0
Postorbital distance	43.1	42.1	1.8	38.4–45.8
Interorbital distance	27.8	25.4	2.5	20.1–30.1
Cheek depth	29.7	29.2	4.5	20.7–37.0
Lower jaw length	43.6	42.6	2.4	35.4–45.7
Preorbital distance	14.4	14.0	1.7	10.3–18.1
Premaxilla dentigerous arm	36.6	35.9	2.5	30.4–39.9
% of caudal peduncle depth				
Caudal peduncle length	94.0	92.5	8.8	77.2–117.6

Meristics	Neotype	Median	Range
Upper lateral-line scales	19	17	16–19
Lower lateral-line scales	10	11	6–13
Total lateral-line scales	29	30	27–31
Circumpeduncular scales	16		16
Dorsal fin spines	14	14	13–15
Dorsal fin rays	12	12	10–12
Anal fin spines	3		3
Anal fin rays	10	10	9–11
Pectoral fin rays	13	14	12–14
Gill rakers (lower limb of first arch)	7	7	6–8
Total gill rakers	11	11	10–13

scales. Head short, with straight to slightly profile, mouth sub-isognathous to prognathous; snout relatively long, eyes of intermediate size, cheek with high number of scale rows in most specimens. Upper jaw with two rows of teeth, lower jaw with one row. Infraorbital series containing a lachrymal with four openings of the laterosensory system and five additional tubular bones. Premaxilla with two, dentary

with one row of regularly set unicuspid teeth. Six to eight gill rakers on ceratobranchials of first outer gill arch, and three to five gill rakers on upper parts.

Scales cycloid. Three or five rows of scales on cheek; four horizontal rows on opercle. Dark spot on outer edge of opercle without scales. Chest-scales mostly smaller than body scales, four or five scales between pectoral and pelvic fins. Upper lateral line separated from dorsal fin base anteriorly by three and a half to four and a half scales, at the 8th pored scale by two and a half or three scales, and at last pored scale by one and a half or two scales. End of upper lateral line rarely overlapping lower lateral line or distanced by no scale, more often separated from beginning of lower lateral line by one to five rows of scales. About 1⁄4 of caudal fin covered with scales, and all other fins unscaled.

Color of living specimens (Fig. 9E, F): No sex-specific coloration differences visible. General color pattern silvery-gray to pale yellow or greenish, with generally five large vertical dark stripes. Scales on flanks of semiadult and adult specimens marked at edges by silvery, pink, or red color with a yellow or golden center, producing a pattern of alternating horizontal silver/red and yellowish/golden lines on the flanks. In adult and possibly dominant (or territorial) specimens, the red lines on lower flank parts may broaden and form a uniform colored red belly part which may extend to lower parts of head. Operculum often with two red spots flanking opposite sides of large black opercular spot, upper spot, or sometimes both red spots may disappear in specimens, possibly depending on behavioral situation or dominant status. Dorsum dark grayish. Dark lachrymal stripe extending over iris and above eye. Fins pale yellowish or whitish in semiadult specimens. In adults dorsal, caudal and anal fins dark gray to blackish, dorsal fin and most upper edge of caudal fin often with thin red margin, pectoral fins with pale yellowish to pale grayish coloration. Outer edge of pelvic fins black, most proximate parts of these fins paler to yellowish. Smallest juveniles (for the first weeks of life) with pale yellow body color and one prominent horizontal black band on body, extending from head to end of caudal peduncle.

Preserved specimens: General body color yellowish-brown with five large dark blotches or stripes on the flanks; less visible in old material. Large black opercular spot. Longitudinal pattern of brown and yellow alternating lines can be visible in some specimens.

Distribution: *Hemichromis angolensis* is widely distributed in southern African river systems: Cuanza, Zambezi River, and Okavango (Fig. 11).

Rubricatochromis gen. nov.

New genus name registered at zoobank.org under lsid:zoobank.org:pub:C5E004F6-03F2-448A-BD65-89EF1439EA69

Type species: *Rubricatochromis guttatus* (Günther, 1862) (Fig. 12).

Included species—herein we only list species that are currently accepted as valid in Daget et al. (1991) and species with population or strain names, that are not assigned to any described species at moment and of which we have used material for the current study:

Rubricatochromis bimaculatus (Gill, 1862)
Rubricatochromis cerasogaster (Boulenger, 1898)
Rubricatochromis cristatus (Loiselle, 1979)
Rubricatochromis guttatus (Günther, 1862)
Rubricatochromis letourneuxi (Sauvage, 1880)
Rubricatochromis lifalili (Loiselle, 1979)
Rubricatochromis paynei (Loiselle, 1979)
Rubricatochromis sp. ‘Gabon’
Rubricatochromis sp. ‘Guinea 1’
Rubricatochromis sp. ‘Guinea 2’
Rubricatochromis sp. ‘neon.’
Rubricatochromis stellifer (Loiselle, 1979).

Diagnosis

Small- to medium-sized cichlids (maximum SL 36.9–85.2 mm), of ovoid body shape with large head and moderately protruding mouth (vs. highly in *Hemichromis*). Teeth in both jaws unicuspid, usually situated in one outer row; unregular inner buccal teeth variably disposed possible in some, mainly bigger specimens. Scales cycloid. 16 scales around caudal peduncle. Vertebrae counts 25–26, with 12–14 abdominal and 11–14 caudal. Infraorbital series containing a lachrymal with four openings of the laterosensory system and five additional tubular bones. Microbranchiospines absent on gill arch 1, but present on outer aspects of gill arches 2 to 4. Caudal skeleton without or with poorly developed hypurapophysis on

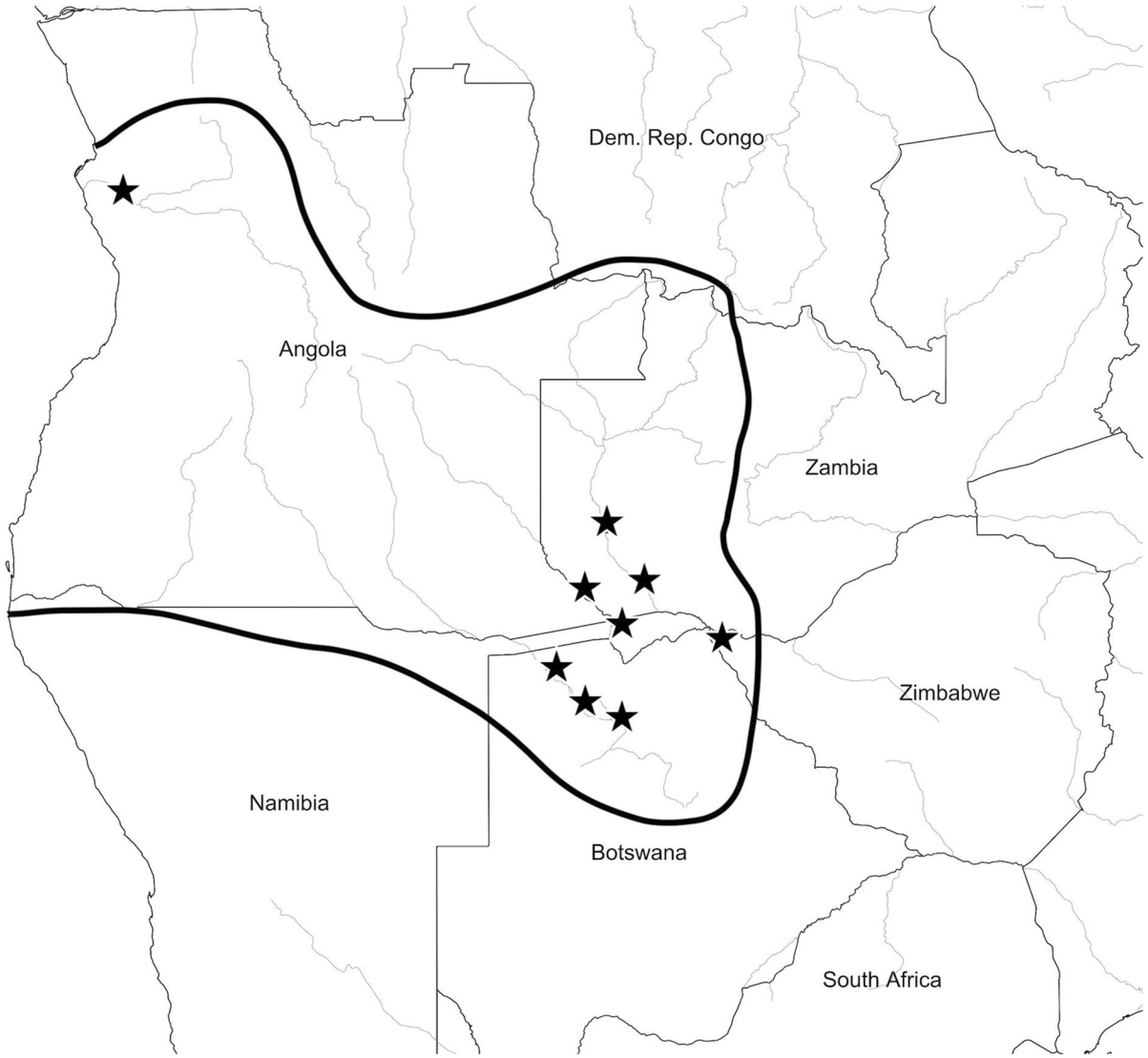

Fig. 11 Map indicating distribution range of *H. angolensis*. Stars indicate collection sites for all specimens used in this study

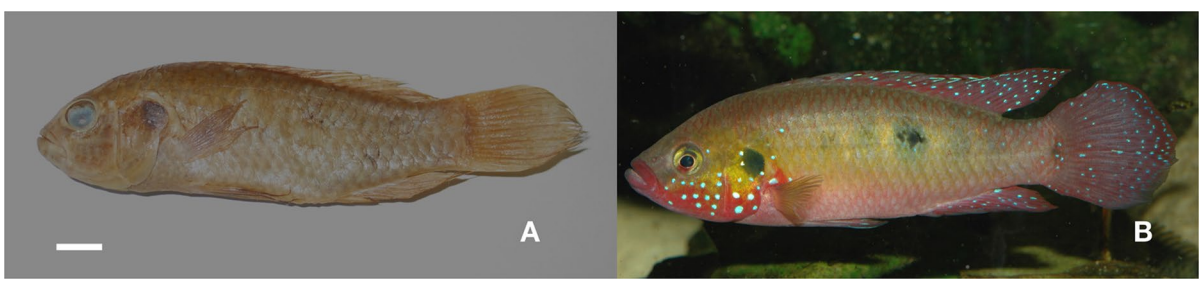

Fig. 12 **A** one of the syntypes of *H. guttatus*, BMHN 1860-4-19-3; SL 91.0 mm, scale bar = 10 mm; **B** life specimen of *H. guttatus* collected from Benin, Oueme River system, in aquarium (coll. L.A., not preserved, not measured)

parhypural. Upper lateral line clearly separated from dorsal fin base. Sexual dichromatism and dimorphism poorly developed. Semiadults and adults usually with three distinct black spots, visible in some behavioral situations, of which the first is on the opercle, the second in middle of body the last on end of caudal peduncle; spots on midbody and caudal peduncle can disappear in most species in several behavioral situations, also in some species depending on age.

All species form pairs and are free substrate spawners with intense brood care.

Distribution: From North Africa to West and Central Africa down to the Congo River basin.

Etymology: From rubricatus (lat) = red colored, refers to the bright red coloration on body of most species when in dominance or broodcare; and chromis—a common ending for African cichlid fishes.

Discussion

Deep divergence in hemichromines and justification for the erection of a new genus

Hitherto the tribe Hemichromini has included only two genera, *Hemichromis* and the monotypic *Anomalochromis*. Even though it has been long recognized that *Hemichromis* comprises two distinct groups, the five-spotted *Hemichromis* and the jewel cichlids (Loiselle, 1979; Greenwood, 1985a, b; Lamboj, 2004), no detailed comparative analysis has been conducted so far to look into the extent of morphological and genetic divergence between these two groups. In the present study, using an integrative approach based on morphology, coloration, and molecular multilocus data, we find strong support for a quite deep divergence between the five-spotted *Hemichromis* and the jewel cichlids. Indeed, the levels of divergence between these two groups at the single-locus level and the inferred divergence time are comparable to that observed among tribes of the Lake Tanganyika cichlid radiation (Breman et al., 2016; Irisarri et al., 2018; Ronco et al., 2021). Together with the observed clear divergence in morphology (and gross color patterns), this justifies the erection of a new genus, *Rubricatochromis*, for the jewel cichlids, which also required a revised diagnosis for *Hemichromis*.

Diversity within *Hemichromis*

Consistent with the recent study by Bitja-Nyom et al. (2021), our multilocus analysis shows that *H. camerounensis* is a species distinct from *H. elongatus* and *H. fasciatus*, and that the population from Lake Bosumtwi in Ghana, previously considered a distinct species, *H. frempongi*, is nested within *H. fasciatus*, justifying their synonymization (Bitja-Nyom et al., 2021). In addition, we found that *H. elongatus* from southern Africa do not group with *H. elongatus* from the Ogooue River in Gabon, the type locality of the species, leading us to revalidate *H. angolensis* for these southern populations. Indeed, despite considerable overlap in the morphospace, significant differences were found among all four species based on both morphometrics and meristics. Specimens from the Cavally River, Ivory Coast, morphologically identified as *H. fasciatus*, represent another quite divergent lineage, but, because of lack of specimens for morphological comparison, we refrain from describing this lineage as a new species.

Interestingly, the phylogenetic position of *H. elongatus* differs between our study and Bitja-Nyom et al. (2021). While in our study *H. elongatus* was resolved as sister species of *H. camerounensis*, it was resolved as sister species of *H. fasciatus* in Bitja-Nyom et al. (2021), a difference that might be attributed to the use of different markers in Bitja-Nyom et al. (2021) and our study. In general, the phylogenetic relationships among the main clades within *Hemichromis* do slightly differ among our different single-locus datasets, suggesting a role of (ancient) incomplete lineage sorting (Takahashi et al., 2001). Despite the slight differences in the single-locus trees (Fig. 2), the phylogenetic relationships inferred from the concatenated dataset (Fig. 3) largely mirror the tree inferred from the mitochondrial, and here especially the COI data. This is not unexpected considering that substitution rates in the mitochondrial genome, and especially in the COI, are at least an order of magnitude higher than in most nuclear genes, including the ones used in this study. Thus, the nuclear genes provide only limited phylogenetic information as compared to the mitochondrial data, and fail to resolve the branching order in parts of the tree (Tanaka et al., 2018). To

account for effects of (ancient) incomplete sorting and lack of resolution due to low nuclear substitution rates, more nuclear loci, ideally genome-scale data, would be required.

Consistent with the significant differences in morphometrics and meristics among species of *Hemichromis*, we observed some differences in live body coloration between some populations of *H. camerounensis* from Nigeria (Niger River system, trade import) and Cameroon (Sanaga River, coll A.L.), and specimens of *H. elongatus* from Gabon. When dominant or in breeding coloration, most specimens of *H. elongatus* which had been observed were exhibiting a bright red coloration on lower body parts with well-developed black spots in both sexes (Fig. 13A, personal obs. A.L.). In the specimens from Nigeria and Cameroon, some few dominant and/or brood-guarding males showed a reversed coloration pattern. In these fishes, the black spots on body and caudal peduncle were absent and substituted by pale, whitish to yellowish-brown blotches. In addition, the lower body parts of these fishes lacked the deep red color, but were blackish to brownish (Fig. 13B). Such reverse coloration has not been observed in specimens of *H. elongatus* till now. Special patterns in breeding coloration of specimens of *H. angolensis* have not yet been documented, but non-breeding coloration does not allow a clear discrimination of *H. camerounensis*, *H. elongatus*, and *H. angolensis* (Fig. 9) and thus appears to be pretty conserved across species.

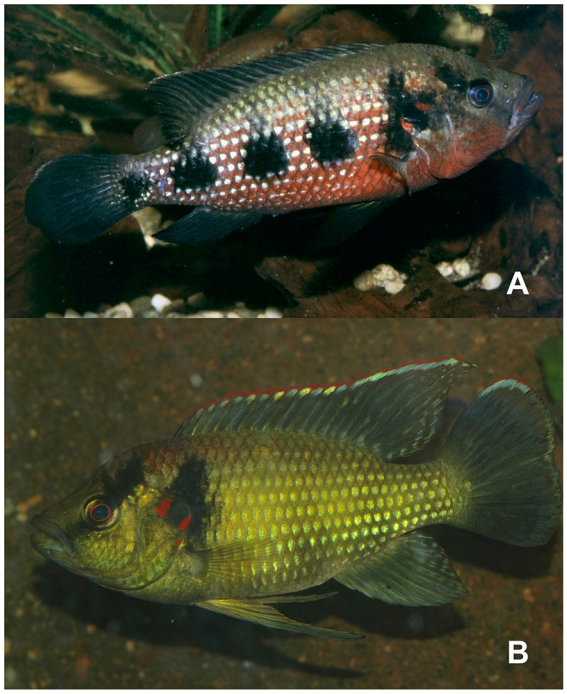

Fig. 13 **A** Adult, dominant male of *H. elongatus* from Gabon, Ogooue River system (pers. coll. A.L.) in aquaria. **B** Adult, dominant male of *H. elongatus* from Cameroon, Mungo River system (pers. coll. A.L.) in aquarium, none preserved, none measured. Notice the loss of the dark spots on the side

The phylogeographic pattern in *Hemichromis* is difficult to reconcile. A principle east (*H. camerounensis* + *H. elongatus*) − west (*H. fasciatus*) divergence is disrupted by *H. angolensis* from southern Africa, which resulted as divergent lineage within *H. fasciatus* in our analyses. In general, the fish fauna in tropical Africa was very much impacted by local extinctions and recolonizations due to climatic fluctuations and river capture events in the Plio- and Pleistocene (e.g., Stewart, 2001), and different taxa show idiosyncratic patterns in their phylogeographic structure across the distribution range of *Hemichromis* (e.g., Goodier et al., 2011; Day et al., 2017; Bragança & Costa, 2019; Van Steenberge et al., 2020; Koblmüller et al., 2021). Yet, the observed pattern in *Hemichromis* is surprising and to some extent unexpected and calls for a more detailed analyses based on multilocus data and a more extensive sampling that covers the whole range of the genus.

Hemichromis, at least some populations, can cope with high salinity. This allows these species to occur in coastal basins and lagoons (Soyinka et al., 2010; Kantoussan et al., 2012) and, potentially, to migrate, at least over short distances, between river estuaries, potentially leading to (low levels of) geneflow among different populations or species, which is not at all uncommon between closely related cichlid species (e.g., Koblmüller et al., 2017; Bradbeer et al., 2019). Indeed, one *H. camerounensis* from the Lagos region in Nigeria, which is close to the western border of the species' distribution range, clustered within *H. fasciatus* in the S7 tree (Fig. 2d), potentially indicating some interspecific geneflow.

Diversity within *Rubricatochromis* gen. nov.

Our DNA sequence data grouped the jewel cichlids into four distinct clades, the phylogenetic placements of which differed depending on the marker analyzed. Some of the species were not resolved as

monophyletic, indicating introgression, recent divergence with incomplete lineage sorting, or a need of synonymization. We further found that the two undescribed species *R.* sp. 'Guinea 1' and *R.* sp. 'Guinea 2' form a distinct clade. Like the species of *Hemichromis*, species of *Rubricatochromis* gen. nov. are highly similar in their overall morphology and usually very difficult to identify, such that species identification in jewel cichlids is typically mainly based on coloration, similar to the situation observed in, e.g., the Lake Tanganyika cichlid genus *Tropheus* (Van Steenberge et al., 2018). Coloration, however, might overlap among currently recognized species, as is the case for *R. guttatus* and *R. letourneuxi* (pers. obs. A.L.). Indeed, as these two species can neither be distinguished based on the DNA sequence data available nor on morphometric and meristic data, their species status is highly questionable. In fact, the only species that can be readily distinguished from its congeners based on anatomical characters is *R. cerasogaster,* in having 2–4 well-developed rows of teeth in both jaws vs. 1–2 in all other species (but if a second row is present, this comprises just one single tooth or very few teeth).

To further discriminate among *Rubricatochromis* species or populations—of which some may represent species of their own—more material is needed from the genus' whole distribution range. As many parts of the distribution ranges for both genera lie in politically highly unstable regions, collecting material, in particular life specimens for behavioral observations and quantification of body coloration, is often difficult to impossible for safety reasons, and hence, geographic sampling is rather sparse, permitting only limited inferences regarding relationships at the intraspecific level or with a phylogeographic scope.

Despite the limited sample size and even though the phylogenetic placements of the four *Rubricatochromis* clades differed depending on the marker analyzed, some limited inferences regarding phylogeography can be drawn from our data. The time tree based on the concatenated data is consistent with an original split between western (Guinea–Kolente) and southeastern *Rubricatochromis* (Gabun and Congo), followed by a progressive expansion of the southeastern *Rubricatochromis* toward the northwest, resulting in the establishment of two further clades, one in Nigeria, and the other widespread in the Upper Guinean and Nilo-Sudanic ecoregions from Guinea-Bissau to Egypt. Especially the spread across the Nilo-Sudanic region happened only very recently, with 12S haplotype sharing between *R. letourneuxi* from Egypt and West African *R. guttatus*. Together with a most recent common ancestor of this entire clade dated to roughly 0.5–1 MYA, this is in line with the inferred recent divergences between West and (North) east African populations/species in some other fishes (e.g., Van Steenberge et al., 2020; Koblmüller et al., 2021). This, however, is not a universal pattern, as some other taxa show considerably deeper divergences across the same geographic scale (e.g., Bragança & Costa, 2019).

Conclusions

In this study, we provided the first phylogenetic analysis of the cichlid tribe Hemichromini, revised the genus *Hemichromis* and erected a new genus *Rubricatochromis* for the jewel cichlids. The genus *Hemichromis* is now restricted to the larger growing, five-spotted cichlids and includes four species, *H. camerounensis*, *H. elongatus*, *H. fasciatus*, and the revalidated *H. angolensis*. However, whether these four species adequately describe the full diversity within the genus remains to be seen, as already with our DNA sequence data and limited sample size we do see some indication for an additional species in West Africa. Together with the study by Bitja-Nyom et al. (2021), this study represents the first steps toward resolving the phylogenetic relationships and taxonomy of the Hemichromini in an integrative approach. To fully understand the diversity within *Hemichromis* and the complex phylogeographic patterns underlying the diversification of the genus, integrative analyses combining phenotypic and large multilocus or ideally genome-scale data from samples covering the genus' entire distribution range are required.

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Data availability Genetic data are available on GenBank (accession numbers listed in Supplementary Table 1) and morphological data are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no conflicts of interests to declare that are relevant to the content of this article.

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